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# The effects of different familial Alzheimer's disease mutations on APP processing in vivo

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## Abstract

**Background:** Disturbed amyloid precursor protein (APP) processing is considered to be central to the pathogenesis of Alzheimer's disease (AD). The autosomal dominant form of the disease, familial AD (FAD), may serve as a model for the sporadic form of AD. In FAD the diagnosis of AD is reliable and presymptomatic individuals carrying FAD mutations can give valuable insights into the earliest stages of the disease where therapeutic interventions are thought to be the most effective.

**Methods:** In the current cross-sectional study, products of APP processing (e.g., sAPP $\alpha$ , sAPP $\beta$ , A $\beta$ <sub>38</sub>, A $\beta$ <sub>40</sub> and A $\beta$ <sub>42</sub>) were measured in the cerebrospinal fluid (CSF) of individuals carrying one of three FAD mutations, *APP*<sup>swe</sup> (p.KM670/671NL), *APP*<sup>arc</sup> (p.E693G) and *PSEN1* (p.H163Y), as well as in non-mutation carriers from the same families.

**Results:** We observed pathological APP processing in presymptomatic carriers of FAD mutations, with different profiles of APP and A $\beta$  isoforms in the three mutation carrier groups, *APP*<sup>swe</sup> (p.KM670/671NL), *APP*<sup>arc</sup> (p.E693G) and *PSEN1* (p.H163Y), except for the well-established decrease in CSF A $\beta$ <sub>42</sub> that was found with all mutations.

**Conclusions:** These findings add to the current evidence that AD pathophysiology differs between disease-causing mutations and can be monitored in the presymptomatic disease stage by CSF analyses. This may also be important from a therapeutic standpoint, by opening a window to monitor effects of disease-modifying drugs on AD pathophysiology.

**Keywords:** Alzheimer's disease, Amyloid precursor protein, Biomarkers, Cerebrospinal fluid, Genetics

## Background

Alzheimer's disease (AD) is a progressive neurodegenerative disorder with pathological hallmarks in the brain, including extracellular amyloid-beta (A $\beta$ ) plaques and intracellular tangles of hyperphosphorylated tau protein. According to the amyloid cascade hypothesis, accumulation of A $\beta$  in the brain is the driver of the disease process [1]. A $\beta$  isoforms ending at amino acid 42 (A $\beta$ <sub>42</sub>) are the most prone to aggregate, both into synaptotoxic oligomers and into fibrils, which are the main components of neuritic plaques [2]. A $\beta$  is a product of the

cleavage of the amyloid precursor protein (APP) by the proteases  $\beta$ -secretase (BACE1) and  $\gamma$ -secretase. Following APP proteolysis at the cell surface, A $\beta$  and sAPP $\beta$ , are shed into the brain interstitial fluid, which communicates freely with the cerebrospinal fluid (CSF). APP can also be cleaved within its A $\beta$  sequence by  $\alpha$ -secretase in a nonamyloidogenic pathway, releasing a different N-terminal APP fragment, sAPP $\alpha$  [3].

The processing of APP is interesting from a therapeutic standpoint because modulation/inhibition at different stages can be feasible to direct APP processing towards the nonamyloidogenic pathway. Measuring the products of APP processing in CSF can clarify how the processing differs in vivo in individuals with AD pathology, compared with healthy controls. This is of particular interest in patients who are still in the

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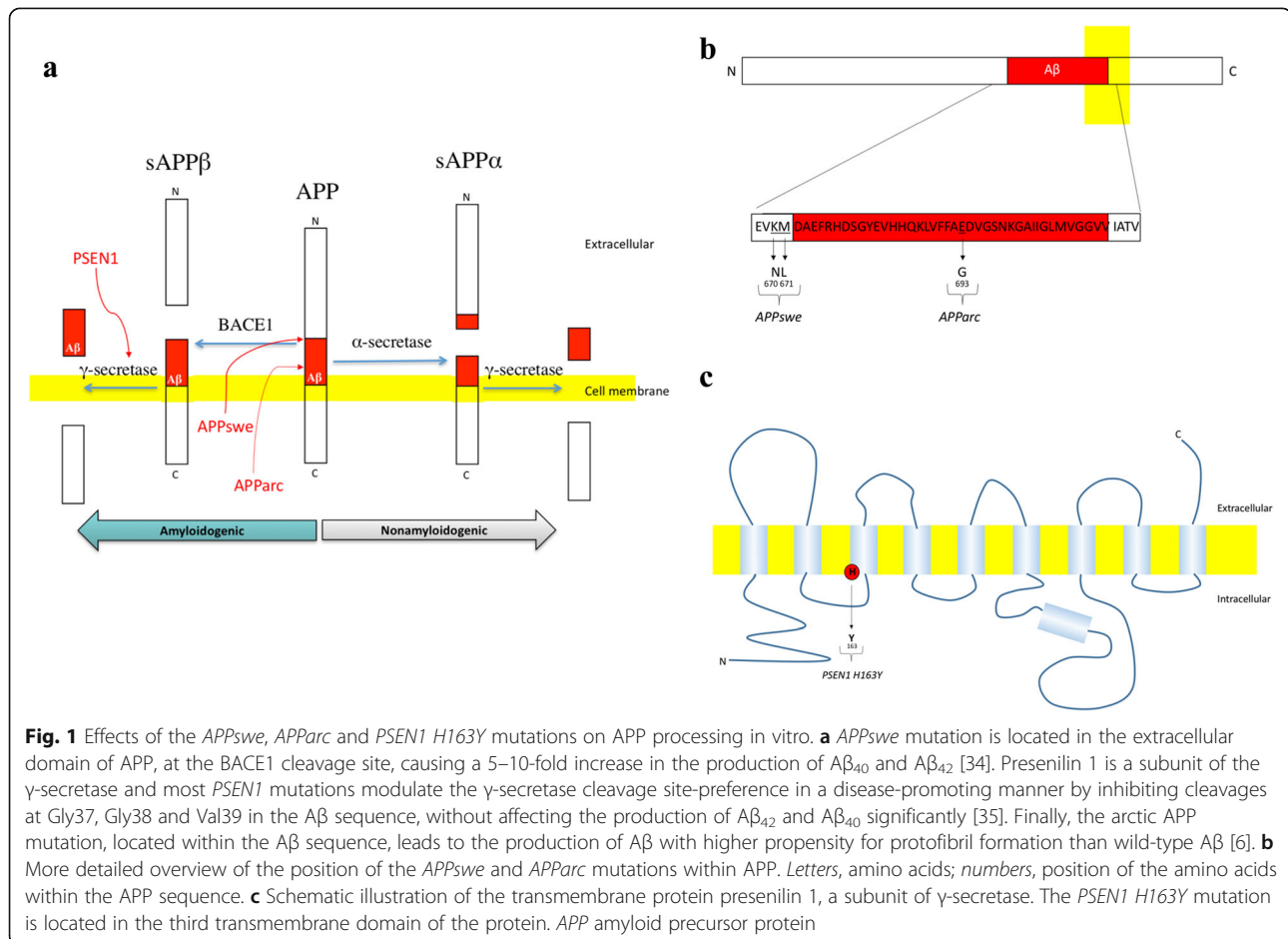
preclinical stage of AD, where disease-modifying drug candidates are most likely to be successful.

To learn more about APP and A $\beta$  metabolism in familial AD (FAD), we measured sAPP $\alpha$ , sAPP $\beta$ , A $\beta$ <sub>38</sub>, A $\beta$ <sub>40</sub> and A $\beta$ <sub>42</sub> in the CSF of individuals carrying autosomal dominant FAD-causing mutations, comparing them with noncarriers from the same families. Most of the subjects were still in the preclinical stage of the disease. We included carriers of three different mutations, *APP*<sup>*swe*</sup> [4, 5], *APP*<sup>*Parc*</sup> [6] and *PSEN1* H163Y [7, 8], each of which causes distinct changes in APP processing in vitro. The effects of the different FAD mutations are illustrated in Fig. 1.

A $\beta$ <sub>42</sub> is a well-established biomarker, which is decreased in the CSF of individuals with AD, most probably due to the accumulation of A $\beta$ <sub>42</sub> in the brain [9]. The same does not apply to CSF A $\beta$ <sub>40</sub>, which has been shown to be unchanged in AD [10–12]. Previous studies have shown conflicting results regarding the levels of other APP processing products in AD. CSF A $\beta$ <sub>38</sub> has been reported unchanged or increased in sporadic AD (SAD) [13, 14] but decreased in FAD [15, 16]. A $\beta$ <sub>16</sub> has been shown to be increased both in SAD and FAD [15, 16]. The levels of

sAPP $\alpha$  and sAPP $\beta$  have been reported to be similar in FAD, SAD and controls [17, 18], but an increase in sAPP $\beta$  has been observed when comparing SAD with frontotemporal dementia [19]. When patients with SAD were compared with patients assessed as having other dementias than AD, the SAD patients had increased levels of both sAPP $\alpha$  and sAPP $\beta$  [20]. In contrast, one study reported a decrease in sAPP $\alpha$  in carriers of the *APP*<sup>*swe*</sup> mutation [21] and another a decrease both in sAPP $\alpha$  and sAPP $\beta$  in patients with advanced AD compared with healthy controls [10].

Here we speculated that the levels of the products of APP processing would already be abnormal in preclinical FAD and differ depending on the underlying mutation: that the carriers of the *APP*<sup>*swe*</sup> mutation would have increased levels of sAPP $\beta$ , A $\beta$ <sub>38</sub> and A $\beta$ <sub>40</sub> as well as decreased levels of sAPP $\alpha$  due to the increased affinity of BACE1 to the mutated APP sequence; that the *APP*<sup>*Parc*</sup> mutation carriers would have normal sAPP $\alpha$  and sAPP $\beta$  levels but decreased levels of A $\beta$ <sub>42</sub>, and potentially A $\beta$ <sub>40</sub> due to fibril formation; and that subjects with the *PSEN1* H163Y mutation would also have normal levels of sAPP $\alpha$  and sAPP $\beta$ , but decreased levels of A $\beta$ <sub>38</sub> as the



mutated  $\gamma$ -secretase preferentially produces  $A\beta_{40}$  and  $A\beta_{42}$ .

In the therapeutic sense it was interesting to see whether these hypotheses would be correct or whether we would find evidence of a common pathological aspect of APP processing in carriers of the different FAD mutations.

Finally, we calculated the sAPP $\beta$ /sAPP $\alpha$  ratio and the  $A\beta_{42}$ / $A\beta_{40}$  ratio, both of which are of interest as potential biomarkers.

## Methods

### Study population

Members of three Swedish families segregating three known mutations leading to autosomal dominant AD, *APP<sup>swe</sup>* (p.KM670/671NL), *APP<sup>arc</sup>* (p.E693G) and *PSEN1* (p.H163Y), were included in the study. The average age at onset of the first clinically relevant symptoms for each family is:  $54 \pm 4$  years for *APP<sup>swe</sup>* (based on 19 affected cases),  $56 \pm 4$  years for *APP<sup>arc</sup>* (based on 12 affected cases) and  $51 \pm 7$  years for *PSEN1 H163Y* (based on 11 affected cases). The average age at onset in each family is normally distributed and is therefore presented as a mean with standard deviation. Participants were recruited to a longitudinal clinical and experimental FAD study through the Genetics Unit, which provides genetic counseling at the Memory Clinic at the Karolinska University Hospital, and were examined between 1993 and 2011. The FAD study is a prospective study including a thorough clinical evaluation, a comprehensive neuropsychological test battery, neuroimaging (3Tesla MRI), electroencephalography and biochemical assessments, including collection of CSF. For AD diagnosis, the criteria for dementia according to the National Institute of Neurological Disorders and Stroke, Washington DC, and Alzheimer Disease and Related Disorders Association, Washington DC (NINCDS-ADRDA) were used [22]; and for the diagnosis of mild cognitive impairment (MCI) we used the criteria of the International Working Group on Mild Cognitive Impairment [23]. In a person with MCI the ability to perform activities of daily living is preserved, while at the same time there is evidence of cognitive decline either objectively measured over time or subjectively reported by the individual/an informant in conjunction with objective deficits. The study subjects are a subsample of individuals at 50% risk of carrying one of the mutations leading to FAD who gave informed written consent to and underwent lumbar puncture ( $n = 36$ ). The CSF was collected at baseline/the entrance of each participant into the study, during the time period of 1993–2011, and the age of the participants presented in the following sections is the age at CSF collection. Of the 36 subjects who underwent the procedure, 18 came from a family

segregating the *APP<sup>swe</sup>* mutation, 10 from a family segregating the *APP<sup>arc</sup>* mutation and eight from a family segregating the *PSEN1 H163Y* mutation. All of the subjects received genetic counseling in conjunction with their participation in the study. The participants, clinicians and researchers involved in the study were blind to the mutation status of the asymptomatic participants unless the participant opted for presymptomatic genetic testing (in a clinical genetics setting). All study procedures were approved by the Regional Ethical Review Board in Stockholm, Sweden.

### Cerebrospinal fluid sampling

CSF samples were collected by lumbar puncture in the L3/L4 or L4/L5 interspace at variable time points during the day. According to current recommendations on CSF sampling and handling, diurnal variation of AD biomarkers should not be a concern [24]. Some of the participants received premedication with 5 mg diazepam and 1 g paracetamol prior to the procedure. Immediately after collection the CSF was centrifuged at  $2200 \times g$  at room temperature for 10 minutes. The supernatant was collected and aliquoted into polypropylene cryotubes. The aliquots used in the current study were stored at  $-80^\circ\text{C}$  and had been thawed and refrozen once before being thawed for analysis in this study.

### Assay of sAPP $\alpha$ , sAPP $\beta$ , $A\beta_{38}$ , $A\beta_{40}$ and $A\beta_{42}$

The CSF samples were all analyzed at the same time by electrochemiluminescence technology (Meso Scale Discovery, Gaithersburg, MD, USA). An analysis of the stability of the frozen CSF samples had been performed previously and did not raise concerns. Concentrations of  $A\beta_{38}$ ,  $A\beta_{40}$  and  $A\beta_{42}$  were analyzed using the MS6000 Human Abeta 3-Plex Ultra-Sensitive Kit (the 6E10 version), while  $\beta$ -secretase cleaved soluble APP (sAPP- $\beta$ ) and  $\alpha$ -secretase cleaved soluble APP (sAPP- $\alpha$ ) in CSF were analyzed using the MS6000 Human sAPPalpha/sAPPbeta Kit, following the recommendations by the manufacturer, and as described previously [25]. Because the *APP<sup>swe</sup>* mutation changes the neo-epitope recognized by the capturing antibody in the sAPP $\beta$  assay, CSF sAPP $\beta$  levels in carriers of the *APP<sup>swe</sup>* mutation were not included in the study.

### Genetic analysis

#### Apolipoprotein E

The *APOE* genotyping was performed for SNPs rs7412 and rs429358 using TaqMan<sup>®</sup> SNP Genotyping Assays (ABI, Foster City, CA, USA) according to the manufacturer's protocol. The amplified products were run on 7500 fast Real-Time PCR Systems (ABI).

### Mutation analyses in *APP* and *PSEN1*

Exons 16 and 17 in *APP* were sequenced to screen for the KM670/671NL and the E693G mutations [12, 14]. To confirm the H163Y mutation in *PSEN1*, exon 6 was sequenced [26]. DNA was amplified using AmpliTaq Gold® 360 PCR Master Mix (Applied Biosystems, Foster City, CA, USA). Primer sequences and PCR conditions are available upon request. The Big Dye® terminator v3.1 Cycle sequencing Kit (Applied Biosystems, Austin, TX, USA) was used for Sanger sequencing. The exons in *APP* and *PSEN1* were sequenced in both directions and analyzed on an ABI3500 Genetic Analyzer (Applied Biosystems, Foster City).

### Statistical analysis

Because of the fact that the age at symptom onset in FAD varies between families, each subject's age relative to the mean age at clinical onset in their respective family was calculated. Unpaired *t* tests and Pearson correlations were applied to normally distributed data, while the Mann–Whitney U test and Spearman correlations were performed on data that was not normally distributed. The distribution of data was assessed with the D'Agostino–Pearson normality test. Thus age, years to predicted family specific clinical onset and CSF biomarker levels were compared between noncarriers of the FAD mutations and the mutation carrier group as a whole as well as the noncarriers and carriers of each of the three mutations separately. Fisher's exact test was used to compare gender and the frequency of the *APOE* ε4 allele between the noncarriers and each of the mutation carrier groups.

Exploratory Pearson/Spearman correlations were performed between biomarker levels, as well as between biomarker levels and predicted age at symptom onset. Two-tailed *p* < 0.05 was considered significant.

## Results

### Demographics of the study population

Of the 36 subjects who underwent a lumbar puncture, 19 were mutation carriers (MC) and 17 were noncarriers (NC). Of the MC, six carried a *PSEN1* H163Y mutation, four carried an *APP*arc mutation and nine carried an

*APP*swe mutation (see Table 1 for a summary of the demographic data of the study population). There was no significant difference between the MC group as a whole, or any of the MC subgroups, and the NC regarding gender distribution, mean age or mean number of years to predicted family specific onset. For the sake of anonymity, the gender distribution in each mutation group is not revealed in Table 1, because this could cause certain participants to be able to deduct their mutation status from the data. The *PSEN1* carriers were somewhat younger than the NC, but this difference did not reach statistical significance. Furthermore, there was no significant difference in the prevalence of *APOE* ε4 carriers between the NC group and the different MC groups and there were only two individuals, a noncarrier and a carrier of the *APP*swe mutation, who were *APOE* ε4 homozygotes. At the time of CSF sampling, three of the *APP*swe carriers fulfilled the diagnostic criteria for AD with dementia, two *APP*swe carriers and one *PSEN1* carrier fulfilled the criteria for MCI and the other 30 subjects (13 MC and 17 NC) were free of symptoms.

### Levels of sAPPα, sAPPβ, Aβ<sub>38</sub>, Aβ<sub>40</sub> and Aβ<sub>42</sub>

There was no significant difference in the levels of sAPPα when comparing the MC group as a whole with the NC. The same applied to sAPPβ when the *PSEN1* and *APP*arc carriers were grouped together and compared with the NC. In contrast, all of the measured Aβ isoforms, Aβ<sub>38</sub>, Aβ<sub>40</sub> and Aβ<sub>42</sub>, were significantly lower in the MC group. When the MC were grouped by their specific mutation, a somewhat different pattern emerged. The carriers of the *APP*swe mutation had significantly lower levels of sAPPα than NC (note that *APP*swe MC were not included in the analysis of CSF sAPPβ because the end-specific capture antibody in the assay will not react with sAPPβ modified at positions 670/671 by the mutation), normal levels of Aβ<sub>38</sub> and Aβ<sub>40</sub> and significantly decreased levels of Aβ<sub>42</sub>. The *APP*arc carriers shared the same pattern of the Aβ isoforms as the *APP*swe carriers, but had normal levels of sAPPα and high sAPPβ levels. Finally, the *PSEN1* carriers had low levels of both Aβ<sub>38</sub> and Aβ<sub>42</sub>, and normal levels of Aβ<sub>40</sub>,

**Table 1** Demographics of the study population

	All MC (n = 19)	<i>APP</i> swe (n = 9)	<i>APP</i> arc (n = 4)	<i>PSEN1</i> (n = 6)	NC (n = 17)
Age (SD)	46.7 (11.9)	51.2 (12.1)	50 (6.6)	33.7 (7.0)	47.8 (10.2)
Years to onset (SD)	−7.7 (12.3)	−1.8 (12.1)	−7 (6.6)	−21.3 (7.0)	−6.9 (9.9)
<i>ApoE</i> ε4 carrier	9/19	5/9	1/4	3/6	7/17
Normal cognition	13/19	4/9	4/4	5/6	17/17
MCI	3	2	-	1	-
AD	3	3	-	-	-

AD Alzheimer's disease, MC mutation carriers, MCI mild cognitive impairment, NC noncarriers



sAPP $\alpha$  and sAPP $\beta$ . See Fig. 2 for a summary of the levels of the APP processing products.

These calculations were repeated after excluding all subjects with MCI or AD diagnosis. This did not change the results for the levels of sAPP $\alpha$ , sAPP $\beta$  and A $\beta_{42}$ , with all of the significant differences in Fig. 2 remaining significant. The levels of A $\beta_{38}$  were no longer significantly lower in the MC group as a whole when subjects with MCI and AD had been excluded, but remained significantly low in the *PSEN1* carrier group. The results for A $\beta_{40}$  remained unchanged after removing MCI and AD subjects, except the A $\beta_{40}$  levels of the *PSEN1* carriers, which were significantly lower than in the NC when the *PSEN1* carrier with MCI diagnosis was excluded.

#### sAPP $\beta$ /sAPP $\alpha$ ratio and A $\beta_{42}$ /A $\beta_{40}$ ratio

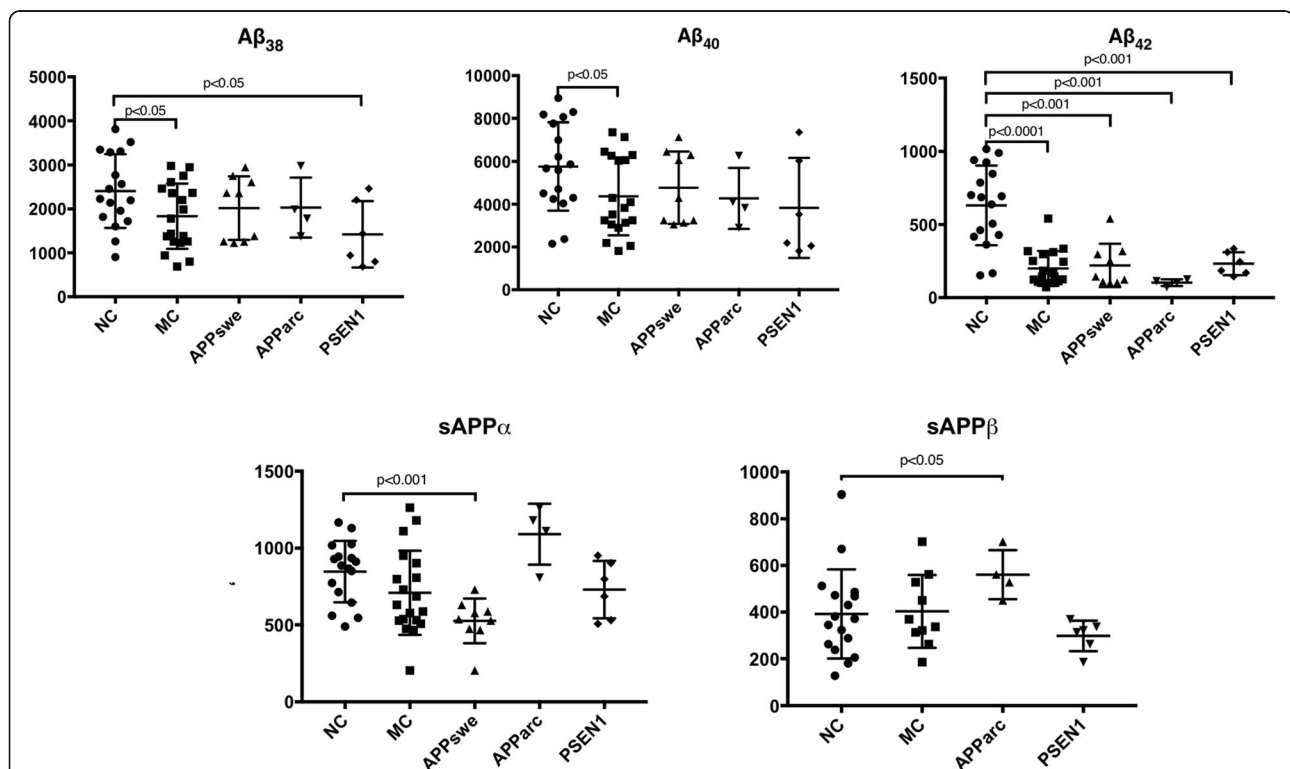
There was no significant difference in the sAPP $\beta$ /sAPP $\alpha$  ratio when comparing the *PSEN1* and *APP<sub>arc</sub>* carriers with the NC, irrespective of whether the NC were compared with the MC as a whole or with a subgroup with a specific mutation. The same applied when the NC were

compared with the presymptomatic *PSEN1* carriers only (data not shown).

The A $\beta_{42}$ /A $\beta_{40}$  ratio was significantly lower in the MC group as a whole than in the NC group (0.05 vs 0.11,  $p < 0.0001$ ). The same applied to all the subgroups, the *APP<sub>swe</sub>* carriers vs the NC (0.04 vs 0.11,  $p < 0.0001$ ), the presymptomatic *APP<sub>swe</sub>* carriers vs the NC (0.05 vs 0.11,  $p = 0.002$ ), the *APP<sub>arc</sub>* carriers vs the NC (0.03 vs 0.11,  $p < 0.001$ ), the *PSEN1* carriers vs the NC (0.07 vs 0.11,  $p < 0.01$ ) and the presymptomatic *PSEN1* carriers vs the NC (0.07 vs 0.11,  $p < 0.01$ ).

#### Correlations between the different products of APP processing

Because of the small size of the *APP<sub>arc</sub>* mutation carrier group ( $n = 4$ ) no correlation calculations were performed involving that group alone. As has been reported previously [27], there was a positive correlation between sAPP $\alpha$  and sAPP $\beta$  in the MC group as a whole (excluding the *APP<sub>swe</sub>* carriers as explained earlier), as well as in the NC group. When looking only at the *PSEN1*



**Fig. 2** CSF levels of sAPP $\alpha$ , sAPP $\beta$ , A $\beta_{38}$ , A $\beta_{40}$  and A $\beta_{42}$  in carriers of three FAD mutations. Scatter plots showing levels of sAPP $\alpha$ , sAPP $\beta$ , A $\beta_{38}$ , A $\beta_{40}$  and A $\beta_{42}$  in carriers of three different mutations leading to FAD compared with healthy family members (NC). The mutation carriers were compared with the NC as one group (MC) and by type of mutation (*APP<sub>swe</sub>*, *APP<sub>arc</sub>* and *PSEN1*). Only carriers of the *APP<sub>arc</sub>* and *PSEN1* mutations are included in the calculation of the mean level of sAPP $\beta$ . The biomarker levels in the NC group were normally distributed, except for sAPP $\beta$ . Thus, the Mann–Whitney U test was used when comparing sAPP $\beta$  between groups. Also, the Mann–Whitney U test was used when comparing the *APP<sub>arc</sub>* and *PSEN1* carriers with the NC due to the small sample sizes of these two MC groups. The unpaired *t* test was used for the rest of the data, except when comparing A $\beta_{42}$  between the NC and MC and comparing sAPP $\alpha$  between the NC and *APP<sub>swe</sub>* (here we used the Mann–Whitney U test because these data were not normally distributed). A $\beta$  amyloid beta, MC mutation carriers, NC noncarriers

carriers, this correlation failed to reach significance, probably due to the small number of individuals in the group.

A strong positive correlation was observed between  $A\beta_{38}$  and  $A\beta_{40}$  in all groups. There was a significant correlation between  $A\beta_{40}$  and  $A\beta_{42}$  as well, in all groups except the *PSEN1* carriers. Interestingly, there was a positive correlation between sAPP $\alpha$  and all the measured  $A\beta$  isoforms in the NC group, but not in any of the mutation carrier groups. Also, the NC group was the only group showing a significant positive correlation between  $A\beta_{38}$  and  $A\beta_{42}$ .

The correlations between sAPP $\alpha$ , sAPP $\beta$ ,  $A\beta_{38}$ ,  $A\beta_{40}$  and  $A\beta_{42}$  are summarized in Table 2.

#### Correlations between sAPP $\alpha$ , sAPP $\beta$ , $A\beta_{38}$ , $A\beta_{40}$ and $A\beta_{42}$ and years to expected symptom onset

Correlations between sAPP $\alpha$ , sAPP $\beta$ ,  $A\beta_{38}$ ,  $A\beta_{40}$ ,  $A\beta_{42}$  and years to expected symptom onset were calculated to see whether the levels of these markers changed as the onset of symptoms approached. No such changes were observed in any of the five markers in the whole MC group, the *PSEN1* group or in the NC group. In the *APPswe* mutation carriers, the concentration of

$A\beta_{38}$  ( $r = -0.69$ ,  $p = 0.04$ ),  $A\beta_{40}$  ( $r = -0.67$ ,  $p = 0.05$ ) and  $A\beta_{42}$  ( $r = -0.86$ ,  $p < 0.01$ ) decreased significantly when the expected symptom onset approached and beyond (see Fig. 3). When subjects with a dementia diagnosis were excluded from the *APPswe* mutation carrier group these correlations failed to reach significance, apart from  $A\beta_{42}$ , which still showed a decrease as age at onset approached ( $r = -0.85$ ,  $p = 0.03$ ). It was not possible to repeat this calculation with only pre-symptomatic *APPswe* mutation carriers because their number is too small.

#### Correction for multiple comparisons

All of the significant correlations presented in Table 2 remained significant after correcting for multiple comparisons using the false discovery rate (FDR) correction. The same applied to the biomarker levels presented in Fig. 2, except for the low  $A\beta_{38}$  levels in the MC and the *PSEN1* groups and the low  $A\beta_{40}$  levels in the MC group which did not survive this correction.

#### Discussion

Compared with noncarriers, carriers of the *APPswe* mutation have significantly decreased CSF levels of

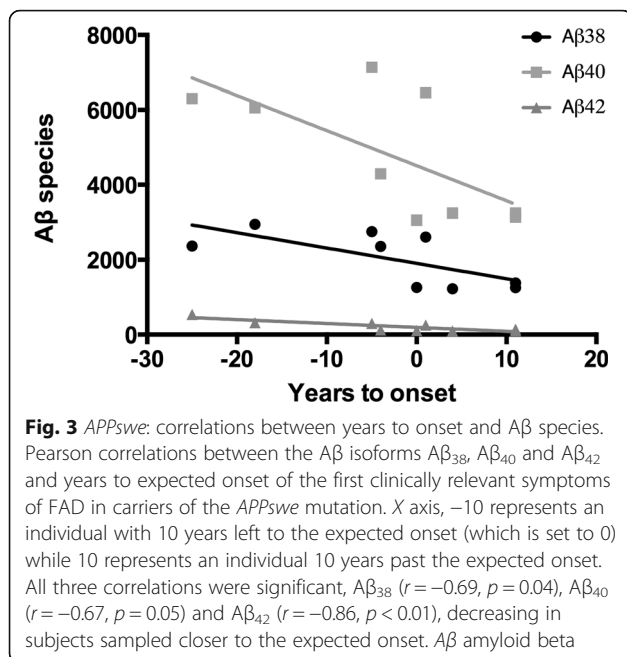
**Table 2** Correlations between the products of APP processing in FAD mutation carriers

	CSF biomarker	sAPP $\alpha$	sAPP $\beta$	$A\beta_{38}$	$A\beta_{40}$	$A\beta_{42}$
MC	sAPP $\alpha$		<i>0.87 (&lt;0.01)</i>	0.2 (0.4)	0.09 (0.7)	-0.2 (0.4)
	sAPP $\beta$	<i>0.87 (&lt;0.01)</i>		0.2 (0.3)	0.1 (0.6)	-0.2 (0.5)
	$A\beta_{38}$	0.2 (0.4)	0.2 (0.3)		<i>0.93 (&lt;0.0001)</i>	0.44 (0.06)
	$A\beta_{40}$	0.09 (0.7)	0.1 (0.6)	<i>0.93 (&lt;0.0001)</i>		<i>0.6 (0.01)</i>
	$A\beta_{42}$	-0.2 (0.4)	-0.2 (0.5)	0.44 (0.06)	<i>0.6 (0.01)</i>	
<i>APPswe</i>	sAPP $\alpha$			0.2 (0.6)	0.2 (0.7)	-0.03 (0.9)
	sAPP $\beta$	n.a.	n.a.	n.a.	n.a.	n.a.
	$A\beta_{38}$	0.2 (0.6)			<i>0.9 (&lt;0.001)</i>	0.7 (0.06)
	$A\beta_{40}$	0.2 (0.7)		<i>0.9 (&lt;0.001)</i>		<i>0.8 (0.01)</i>
	$A\beta_{42}$	-0.03 (0.9)		0.7 (0.06)	<i>0.8 (0.01)</i>	
<i>PSEN1</i>	sAPP $\alpha$		0.66 (0.18)	0.20 (0.7)	0.20 (0.7)	0.086 (0.9)
	sAPP $\beta$	0.66 (0.18)		0.60 (0.2)	0.60 (0.2)	0.54 (0.3)
	$A\beta_{38}$	0.20 (0.7)	0.60 (0.2)		<i>1 (&lt;0.01)</i>	0.83 (0.06)
	$A\beta_{40}$	0.20 (0.7)	0.60 (0.2)	<i>1 (&lt;0.01)</i>		0.83 (0.06)
	$A\beta_{42}$	0.086 (0.9)	0.54 (0.3)	0.83 (0.06)	0.83 (0.06)	
NC	sAPP $\alpha$		<i>0.64 (&lt;0.01)</i>	<i>0.71 (&lt;0.01)</i>	<i>0.72 (&lt;0.01)</i>	<i>0.65 (&lt;0.01)</i>
	sAPP $\beta$	<i>0.64 (&lt;0.01)</i>		0.28 (0.3)	0.33 (0.2)	0.29 (0.3)
	$A\beta_{38}$	<i>0.71 (&lt;0.01)</i>	0.28 (0.3)		<i>0.98 (&lt;0.0001)</i>	<i>0.95 (&lt;0.0001)</i>
	$A\beta_{40}$	<i>0.72 (&lt;0.01)</i>	0.33 (0.2)	<i>0.98 (&lt;0.0001)</i>		<i>0.98 (&lt;0.0001)</i>
	$A\beta_{42}$	<i>0.65 (&lt;0.01)</i>	0.29 (0.3)	<i>0.95 (&lt;0.0001)</i>	<i>0.98 (&lt;0.0001)</i>	

Correlations between the products of APP processing in the mutation carrier group as a whole (MC), carriers of the *APPswe* and *PSEN1* mutations separately and in the nonmutation carriers (NC)

Numbers represent Pearson/Spearman correlation coefficients ( $p$  values) (significant correlations in italics)

APP amyloid precursor protein, FAD familial Alzheimer's disease,  $A\beta$  amyloid beta, MC mutation carriers, NC noncarriers



sAPPα and Aβ<sub>42</sub> whereas the levels of Aβ<sub>38</sub> and Aβ<sub>40</sub> were not significantly different. The decreased levels of Aβ<sub>42</sub> are expected, because Aβ<sub>42</sub> is a well-established AD biomarker and has been shown to be decreased in AD patients in numerous studies [9]. The sAPPα levels have also been shown to be reduced in a previous study on some of the same individuals [21]. This could be due to the increased activity of the amyloidogenic pathway of APP processing in *APPswe* carriers at the expense of the nonamyloidogenic pathway in which sAPPα is produced.

In the *PSEN1* carriers the CSF levels of sAPPα and sAPPβ were unchanged, whereas sAPPβ was increased in the *APParc* carriers. The *APParc* carriers also showed the expected decrease in Aβ<sub>42</sub> levels, while both Aβ<sub>38</sub> and Aβ<sub>42</sub> were decreased in the *PSEN1* carriers. The decrease in Aβ<sub>38</sub> has been observed in previous studies on *PSEN1* carriers [15, 16] and the same applies to the normal levels of sAPPα and sAPPβ in the *PSEN1* carriers [17, 18]. Amyloid plaques including Aβ<sub>38</sub> deposits, have been shown to be limited to FAD cases and this accumulation of Aβ<sub>38</sub> in the brain could explain the low levels Aβ<sub>38</sub> in CSF [28]. Another, possibly coexisting, explanation could be that the mutated γ-secretase preferentially produces Aβ<sub>40</sub> and Aβ<sub>42</sub>, at the expense of Aβ<sub>38</sub>. The decrease in Aβ<sub>38</sub> levels seems quite robust, remaining significant when only presymptomatic *PSEN1* carriers were included. In contrast, the observed reduced levels of Aβ<sub>38</sub> for the whole MC group were no longer significantly low when looking only at presymptomatic individuals. Taken together our results emphasize the differences in production and metabolism of Aβ isoforms in different FAD mutations. The results for the

*PSEN1* and *APParc* carriers fit our hypothesis quite nicely, apart from the observed increased levels of sAPPβ in the *APParc* carriers. A possible explanation for this observation might be that increased fibril formation and lack of monomeric Aβ isoforms somehow stimulates amyloidogenic APP processing.

When looking at correlations between years to symptom onset and the different APP processing products it is interesting to see that the products remain stable in subjects at different time points from expected onset in the MC group as a whole and in the *PSEN1* carriers alone. The Aβ isoforms remained low in the MC compared with the NC, while there was no difference in the levels of sAPPα and sAPPβ in the MC and the NC. Very few studies have looked at APP processing in preclinical FAD [15–17] and to our knowledge none have correlated the APP processing products to years to symptom onset.

According to one study on SAD, there were no differences in BACE1 activity, sAPPα, sAPPβ and Aβ<sub>40</sub> concentrations between AD patients and controls until the levels of all of these biomarkers dropped in advanced stages of AD [10]. The only mutation carrier group in the current study that showed a change in these biomarkers in relation to expected symptom onset was the *APPswe* group, in which the levels of all of the Aβ isoforms decreased with progression of the disease. This resonates quite well with the study by Rosen et al. [10] on SAD, because the *APPswe* mutation carriers were the only group including individuals that already had a dementia diagnosis. The correlation ceased to be significant when the subjects with dementia were excluded, apart from Aβ<sub>42</sub>, which still showed a decrease over time. A longitudinal study on the subjects included here is ongoing and may shed light on the changes in these markers over time through repeated sampling from the same individuals.

We replicated the previously observed positive correlation between sAPPα and sAPPβ in both the MC group (excluding the *APPswe* carriers) and the NC group. This observation correlates well with other studies including healthy individuals as well as subjects with MCI, FAD and SAD, suggesting that the α and β pathways are noncompetitive [17, 20, 26, 27, 29, 30]. This positive correlation also applies to the MC in this study, possibly excluding the *APPswe* carriers because they had low levels of sAPPα. Because of limitations in the sAPPβ assay we do not have information on the levels of sAPPβ in the *APPswe* carriers and therefore can only speculate on the correlation between sAPPα and sAPPβ in this group. It is interesting that all three Aβ isoforms are positively correlated to sAPPα but not to sAPPβ in the NC group, as sAPPα is not a product of the same APP cleaving pathway as Aβ. A possible explanation would be that all

of the measured A $\beta$  isoforms are more abundant in the CSF of healthy individuals with high levels of sAPP $\alpha$ , as the threshold for A $\beta$  aggregation in the brain has not yet been reached. A $\beta_{38}$  and A $\beta_{40}$  have a positive correlation to each other and the same is true for A $\beta_{40}$  and A $\beta_{42}$ . This suggests that the A $\beta_{42}$  decrease in CSF in FAD may be accompanied by a decrease in A $\beta_{40}$  and possibly in A $\beta_{38}$  as well, although the current study failed to show a significant correlation between A $\beta_{42}$  and A $\beta_{38}$ .

Finally, we did not observe a difference in the sAPP $\beta$ /sAPP $\alpha$  ratio between the MC and the NC. The sAPP $\beta$ /sAPP $\alpha$  ratio has been suggested as a biomarker for brain amyloidosis [30], but our findings do not support it as a marker of early pathology. This should be interpreted with caution, however, because the possibility remains that the results were negative due to the small sample size in this instance (only 10 MC from the *PSEN1* and *APP $\Delta$ rc* families because data on sAPP $\beta$  were not included from the *APP $\Delta$ swe* MC). As expected, the A $\beta_{42}$ /A $\beta_{40}$  ratio was low in the MC group as a whole and in the MC subgroups compared with the NC. This difference remained statistically significant when symptomatic individuals had been excluded, supporting its role as a robust early AD biomarker.

The method used in the current study to measure the potential biomarkers sAPP $\beta$  and sAPP $\alpha$  has recently been analytically validated using novel standard operating procedures developed within the BIOMARKAPD project [31]. This validation increases the credibility of the results obtained with this method and decreases the likelihood of analysis bias.

The results discussed were not corrected for multiple comparisons and the differences observed in A $\beta_{38}$  and A $\beta_{40}$  levels between groups did not survive a FDR correction. When interpreting the results of the FDR correction one should keep in mind that the study was hypothesis driven and the biomarkers carefully selected before any statistical analysis was performed. Therefore, the study design itself reduces the likelihood of false positives. Also, the small sample sizes, which often require nonparametric testing, already reduce the power to detect true differences between groups. The possibility still remains that the differences observed in A $\beta_{38}$  and A $\beta_{40}$  levels between groups were significant by chance, as suggested by the FDR correction. However, we feel that this possibility is not great enough to disregard the results altogether and that they warrant further study in larger cohorts.

A limitation to this study is the number of included subjects and this should be taken into account when interpreting the results. The current study, however, is larger than previous studies on other aspects of APP processing which have included FAD subjects and replicates some of their results, underlining the robustness of the data acquired from this population. Even though the

small sample size might have had some effect on the outcomes presented here, they are at the very least strongly hypothesis generating. Another limitation is the cross-sectional design of the study, which makes it impossible to assess changes in APP processing products over time. This will be addressed in a longitudinal study on the same subjects, which is ongoing. The usefulness of FAD subjects as models for SAD has been supported by several studies [32], but the possibility still exists that these results might not be generalizable to SAD.

## Conclusion

We observed pathological APP processing in presymptomatic carriers of FAD mutations, which adds to the current evidence that AD pathogenesis is present before the development of clinical symptoms [33]. We found no apparent common aspect of APP processing in the three mutation carrier groups, apart from the well-known decrease in CSF A $\beta_{42}$ , which is an early event in AD pathogenesis. Perhaps this decrease is secondary to the aggregation of brain amyloid, rather than a result of the APP processing steps, and thus a poor marker for the primary etiology. If the same type of treatment was to be used in presymptomatic FAD mutation carriers regardless of the type of mutation, these results suggest that a treatment interfering with the final product of APP processing, A $\beta$ , would be a feasible option.

## Abbreviations

AD: Alzheimer's disease; APP: Amyloid precursor protein; CSF: Cerebrospinal fluid; FAD: Familial Alzheimer's disease; FDR: False discovery rate; MC: Mutation carrier; MCI: Mild cognitive impairment; MRI: Magnetic resonance imaging; NC: Noncarrier; PCR: Polymerase chain reaction; SAD: Sporadic Alzheimer's disease; SD: Standard deviation

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## Availability of data and materials

The datasets generated and analyzed during the current study are not publicly available due to individual privacy of the study participants. However, they are available from the corresponding author upon reasonable request.



### Authors' contributions

ST contributed to the conception and design of the study, acquired the cerebrospinal fluid and clinical data and analyzed and interpreted them. ST drafted the manuscript. AKS was involved in the conception and design of the study, the analysis and interpretation of data and in revising the manuscript critically for intellectual content. OA contributed to the conception and design of the study, acquired neuropsychological data and revised the manuscript critically for intellectual content. HT was responsible for analysis and interpretation of data and for critical revision of the manuscript for intellectual content. ME was involved in the conception and design of the study, the analysis and interpretation of data and in revising the manuscript critically for intellectual content. HZ was involved in the conception and design of the study, the analysis and interpretation of data and in revising the manuscript critically for intellectual content. KB was involved in the conception and design of the study, the analysis and interpretation of data and in revising the manuscript critically for intellectual content. CG was principal investigator in the conception and design of the study, the acquisition of data, the analysis and interpretation of data and in revising the manuscript critically for intellectual content. All authors have given final approval of the version to be published. All authors have agreed to be accountable for all aspects of the work ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

### Competing interests

The authors declare that they have no competing interests.

### Consent for publication

Not applicable.

### Ethics approval and consent to participate

All study procedures were approved by the Regional Ethical Review Board in Stockholm, Sweden. Informed, written consent was obtained from all participants prior to their participation in the study.

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